Conclusions: Bovine SF from joints of healthy calves contains active aggrecanases that are capable of cleaving both adult human and immature bovine aggrecan in the IGD and in the CS2 region. Human SF from knees of adult injured patients, has the same ability to cleave in the CS2 region of both immature bovine and adult human aggrecan, but may have less activity against the bovine aggrecan IGD site. The cleavage seen in the IGD and the CS2 region of bovine aggrecan after incubation with JC is a delayed process that may involve additional time for diffusive transport of aggrecanases out of the JC, as well as time needed for induction or activation of the aggrecanases.

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EVALUATING CATHEPSIN ACTIVITY AND SIRT1 CLEAVAGE IN EXPERIMENTAL OSTEOARTHRITIS

L. Ben-Aderet, G. Blum, M. Dvir-Ginzberg. *Hebrew Univ. of Jerusalem, Jerusalem, Israel*

Purpose: SIRT1 is a NAD-dependent protein deacetylase that regulates cartilage matrix gene expression and was found to be impaired in protein levels and enzymatic activity in OA vs. normal cartilage. Stimulation of chondrocytes with pro-inflammatory factors induces site-specific cleavage of full-length SIRT1 (110kDa) to generate an inactive variant (75SIRT1;:75kDa). This research aims to profile variations in SIRT1 cleavage fragments and cathepsin B and S activities to determine the inflammatory and catabolic state of cartilage and susceptibility to develop Osteoarthritis (OA).
Methods: OA derived articular chondrocytes were obtained from total knee arthroplasty. Following isolation and propagation, primary human chondrocytes where either plated (2D) or encapsulated in three dimensional (3D) alginate micro-beads and cultured in DMEM culture media for 2-weeks. Chondrocytes were treated or untreated with 2 ng/ml IL-1β and 50 ng/ml TNF-α for 24h. Following stimuli, cell extracts and media were obtained and analyzed for SIRT1 cleavage or for cathepsin activity using activity based probes (ABPs), which covalently bind active cathepsin B and S and exert a fluorescent signal. Further, media from round 4-mm-diameter human articular cartilage explants, subjected to cyclic mechanical loading (60 N, 0.1 Hz, 1 h) in the presence or absence of 2 ng/ml IL-1ß and 50 ng/ml TNF-α, were analyzed similarly. Finally, regions of intact cartilage (IC) vs. degenerative cartilage (DC) were cryo-sectioned and stained with fluorescent ABPs to detect temporal changes in cathepsin B and S activities as a function of articular cartilage degeneration.
Results: Chondrocyte lysates from monolayer 2D and 3D cultures, exhibited reduced protein levels of full length SIRT1 (110kDa), with enhanced levels of 75SIRT1 and a :35kDa SIRT1-responsive fragment, when subject to proinflammatory stimuli. Conditioned media from 2D chondrocytes exhibited 3-fold enhancement in 75SIRT1 and :35kDa SIRT1-responsive fragment with full-length SIRT1 being beneath the limit of detection, under proinflammatory conditions. These data correlate with enhanced mRNA expression levels of cartilage degrading enzymes upon cytokine stimuli in both 2D and 3D chondrocytes (i.e. average increase of 160-fold for MMP13; 3-fold for ADAMTS5, 6-fold for cathepsin B, and 50-fold for cathepsin S), and reduced expression for cartilage structural genes (i.e. average reduction of 2-fold for both COL2A1 and ACAN). Analysis of cathepsin activity with ABPs in cell lysates, showed 2-5 fold enhancement of enzymatic activity of cathepsin S with insignificant changes in cathepsin B activity. In addition, conditioned media obtained from plated chondrocytes showed enhanced cathepsin S activity with undetected cathepsin B activity. While both cathepsins showed augmented activity in freshly isolated chondrocytes from IC and DC, surprisingly, only cathepsin S was observed in supernatant media following collagenase treatment in DC regions of OA cartilage, indicating active cathepsin S is secreted by chondrocytes and contributes to extracellular matrix degradation.
Conclusions: In summery, our in-vitro data indicate that the 75SIRT1 fragment is augmented in lysates and conditioned media of plated chondrocytes under proinflammatory conditions. As well, cathepsin activity, especially cathepsin S, is augmented in chondrocytes, subject to proinflammatory stimuli. These data are further supported by the enhanced activity of cathepsin S in degenerated cartilage matrix. Hence these findings may provide a basis for early detection of OA susceptible individuals, based on enhanced cathepsin activity and SIRT1 cleavage, generated in regions of articular cartilage prone to damage.

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DEPLETION OF SIRT6 CAUSES CELLULAR SENESCENCE, DNA DAMAGE, AND TELOMERE DYSFUNCTION IN HUMAN CHONDROCYTES

K. Nagai, T. Matsushita, T. Matsuzaki, K. Takayama, A. Uefuji, S. Zhang, N. Nakano, T. Matsumoto, R. Kuroda, M. Kurosaka. *Dept. of Orthopaedic Surgery, Kobe Univ. Graduate Sch. of Med., Kobe, Japan*

Purpose: Recently, it has been suggested that chondrocyte senescence plays an important role in the pathogenesis and development of Osteoarthritis (OA). SIRT6 is a member of sirtuin family of NAD+-dependent protein deacetylase. Recently, SIRT6 has been implicated in DNA repair, telomere maintenance, attenuation of inflammation and glucose homeostasis. Notably studies in mice have shown that SIRT6 deficiency produces premature aging phenotypes, whereas SIRT6 overexpression causes a moderate increase in the lifespan of male mice, strongly suggesting an important role of SIRT6 in aging and aging-associated diseases. However, its role in chondrocytes has so far not been explored. The purpose of this study is to examine the role of SIRT6 in human chondrocytes by inhibiting SIRT6 with a RNA interference (RNAi) technique. The hypothesis of the present study is that SIRT6 has a protective function toward human chondrocytes
Methods: OA cartilage tissues were obtained from varus knee OA during total knee arthroplasty. Lateral and medial femoral condyles were used as cartilage with mild and severe OA, respectively. Articular cartilage tissues without OA were obtained from the patients underwent surgery for femoral neck fracture. These were used for histological experiments. Next, SIRT6 was depleted by RNAi and examined the influence of SIRT6 depletion on gene expression changes, proliferation, and senescence in normal human chondrocytes. Normal Human Articular Chondrocytes-knee (NHAC-kn) was used as a normal human chondrocytes. RNA or proteins were extracted 48 hours after the lipofection, and real-time PCR and western blotting analysis were respectively performed. The proliferation activity was examined using a Cell Counting Kit-8. Optical density was measured at 0, 24, 48, 72, 96 hours after the lipofection. Senescence associated β-Galactosidase (SA-β-Gal) staining was examined using a SA-β-Gal staining kit. Furthermore, to detect DNA damage and telomere dysfunction, the γH2AX foci and telomere dysfunction-induced foci (TIFs) where γH2AX foci co-localized with telomere repeat binding factor-1 (TRF-1) were examined using an immunofluorescence confocal microscopy.
Results: Immunohistochemical analysis showed that SIRT6-positive cells were observed in normal cartilage, and mild and severe OA cartilage. In real-time PCR analysis, the depletion of SIRT6 significantly increased expression of matrix metalloproteinase (MMP) -1 and 13 mRNA. The proliferation assay showed that the absorbance was significantly decreased by the depletion of SIRT6 at 72 and 96 hours after lipofection, indicating reduced proliferation by the depletion of SIRT6. SA-β-Gal assay showed that the percentage of SA-β-Gal positive cells was significantly increased by the depletion of SIRT6, indicating that the depletion of SIRT6 induced premature senescence. Immunofluorescence microscopic analysis showed that γH2AX foci and TIFs was significantly increased in the SIRT6-depleted chondrocytes compared with control. Furthermore, to examine the downstream signaling pathways that mediate the induction of senescence by the depletion of SIRT6, the protein level of mediators for DNA damage induced-senescence, p16 and p21, was examined by western blotting. p16 protein level was increased while p21 protein level was reduced in the in SIRT6-depleted chondrocytes compared with control.
Conclusions: The present study demonstrated that the depletion of SIRT6 in human chondrocytes caused increased expression of MMP-1 and 13, reduced proliferation and increased senescent cells. In addition, the depletion of SIRT6 caused accumulation of γH2AX and TIFs. These observations suggested that the depletion of SIRT6 induced premature senescence associated with a secretory phenotypic change and that the induction of the premature senescence may be caused by increased DNA damages and telomere dysfunction. Moreover, we observed p16 was increased by SIRT6 depletion. The p16-pRB pathway has been reported to be a major pathways that mediate premature senescence and proliferation arrest. Therefore taken together, these observations suggest the depletion of SIRT6 in human chondrocytes caused DNA damage and telomere dysfunction, followed by a premature senescence possibly via the p16-pRB pathway. SIRT6 may play an important role to protect chondrocytes from premature senescence, DNA damage, and telomere dysfunction.